U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 USC 371 and 37 CFR 1.491

ATTORNEY DOCKET NO.
213373

U.S. APPLICATION NO.
09/936449

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US00/06482	10 March 2000	12 March 1999

TITLE OF INVENTION
METHOD OF INHIBITING A CHAPERONE PROTEIN

APPLICANT(S) FOR DO/EO/US
Marcu et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 USC 371 and 37 CFR 1.491.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 USC 371 and 37 CFR 1.491.

3. ☒ This is an express request to begin national examination procedures (35 USC 371(f)).

4. ☒ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).

5. ☒ A copy of the International Application as filed (35 USC 371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☐ An English language translation of the International Application as filed (35 USC 371(c)(2)).

7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 USC 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.

8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 USC 371(c)(3)).

9. ☐ An oath or declaration of the inventor(s) (35 USC 371(c)(4)).

10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 USC 371(c)(5)).

11. Nucleotide and/or Amino Acid Sequence Submission
   a. ☐ Computer Readable Form (CRF)
   b. Specification Sequence Listing on:
      i. ☐ CD-ROM or CD-R (2 copies); or
      ii. ☐ Paper Copy
   c. ☐ Statement verifying identity of above copies

Items 12 to 19 below concern other document(s) or information included:

12. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
   ☐ Form PTO-1449
   ☐ Copies of Listed Documents

13. ☐ An assignment for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

14. ☒ A FIRST preliminary amendment.
   ☐ A SECOND or SUBSEQUENT preliminary amendment.

15. ☐ A substitute specification.

16. ☐ A change of power of attorney and/or address letter.

17. ☒ Application Data Sheet Under 37 CFR 1.76

18. ☒ Return Receipt Postcard

19. ☒ Other items or information:
   Amendments to Specification made via Preliminary Amendment
   Amendments to Claims made via Preliminary Amendment
   Pending Claims after Entry of Preliminary Amendment

U.S. APPLICATION NO. 09/936449	INTERNATIONAL APPLICATION NO. PCT/US00/06482	ATTORNEY DOCKET NO. 213373

20. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1,000.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... $ 860.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO, but international search fee (37 CFR 1.445(a)(2))paid to USPTO ..... $ 710.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... $ 690.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1) to (4) ..... $ 100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT=	$860.00	
Surcharge of $130.00 for furnishing the National fee or oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date	$	

CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE		
Total Claims	23	-20=	3	x $ 18.00	$54.00	
Independent Claims	1	- 3 =	0	x $ 80.00	$ 0.00	
☐ Multiple Dependent Claim(s) (if applicable)				+$270.00	$	

TOTAL OF ABOVE CALCULATIONS=	$914.00	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.	$	
SUBTOTAL=	$914.00	
Processing fee of **$130.00** for furnishing English Translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date.	$	
TOTAL NATIONAL FEE=	$914.00	
Fee for recording the enclosed assignment. The assignment must be accompanied by an appropriate cover sheet. **$40.00** per property +	$	
TOTAL FEE ENCLOSED=	$914.00	
	Amount to be: refunded	$
	charged:	$

a. ☐ A check in the amount of $914.00 to cover the above fee is enclosed.

b. ☒ Please charge Deposit Account No. 12-1216 in the amount of $914.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 12-1216. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Customer Number: 23460

23460

PATENT TRADEMARK OFFICE

Carol Larcher

Carol Larcher, Registration No. 35,243
One of the Attorneys for Applicants

Date: September 12, 2001

PATENT
Attorney Docket No. 213373

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Marcu et al.

Art Unit: Unassigned

Application No. Unassigned

Examiner: Unassigned

Filed: September 12, 2001

For: METHOD OF INHIBITING A CHAPERONE PROTEIN

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to the examination of the above-identified patent application, please enter the following amendments and consider the following remarks.

AMENDMENTS

IN THE SPECIFICATION:

At page 1, line 2, please insert the following paragraph:

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

This patent application is the U.S. National Phase of PCT/US00/06482 filed on March 10, 2000, which claims priority to U.S. provisional patent application no. 60/124,135 filed on March 12, 1999.

IN THE CLAIMS:

Replace the indicated claims with:

14. (Amended) The method of claim 1, wherein the chaperone protein is in a cell and cellular proliferation is inhibited.

15
16. (Amended) The method of claim 1, wherein the client protein is hepatitis B virus reverse transcriptase.

16
18. (Amended) The method of claim 1, wherein the client protein is a steroid hormone receptor.

17
20. (Amended) The method of claim 1, wherein the client protein is in a cell and is Hsf-1.

18
22. (Amended) The method of claim 1, which is *in vivo*.

19
23. (Amended) The method of claim 1, which is *ex vivo*.

REMARKS

The specification has been amended to incorporate the claim of priority. The claims of the present application have been amended by converting the multiply-dependent claims into singly dependent claims, and by correcting the claim dependencies as necessary. No new matter has been added by way of the foregoing amendments.

Conclusion

The application is considered to be in good and proper form for allowance, and the Examiner is respectfully requested to pass this application to issue. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

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Date: September 12, 2001

PATENT
Attorney Docket No. 213373

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Marcu et al.

Art Unit: Unassigned

Application No. Unassigned

Examiner: Unassigned

Filed: September 12, 2001

For: METHOD OF INHIBITING A CHAPERONE PROTEIN

AMENDMENTS TO SPECIFICATION MADE VIA PRELIMINARY AMENDMENT

The following paragraph has been inserted at page 1, line 2:

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

This patent application is the U.S. National Phase of PCT/US00/06482 filed on March 10, 2000, which claims priority to U.S. provisional patent application no. 60/124,135 filed on March 12, 1999.

PATENT
Attorney Docket No. 213373

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Marcu et al.

Application No. Unassigned

Filed: September 12, 2001

For: METHOD OF INHIBITING A CHAPERONE PROTEIN

Art Unit: Unassigned

Examiner: Unassigned

AMENDMENTS TO CLAIMS MADE VIA PRELIMINARY AMENDMENT

Amendments to existing claims:

14. (Amended) The method of [any of claims 1-13] claim 1, wherein the chaperone protein is in a cell and cellular proliferation is inhibited.

16. (Amended) The method of [any of claims 1, 3-6, 12, and 13] claim 1, wherein the client protein is hepatitis B virus reverse transcriptase.

18. (Amended) The method of [any of claims 1, 3-6, 12 and 13] claim 1, wherein the client protein is a steroid hormone receptor.

20. (Amended) The method of [any of claims 1, 3-6, 12 and 13] claim 1, wherein the client protein is in a cell and is Hsf-1.

22. (Amended) The method of [any of claims 1-21] claim 1, which is *in vivo*.

23. (Amended) The method of [any of claims 1-21] claim 1, which is *ex vivo*.

PATENT
Attorney Docket No. 213373

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Marcu et al.

Application No. Unassigned

Filed: September 12, 2001

For: METHOD OF INHIBITING A CHAPERONE PROTEIN

Art Unit: Unassigned

Examiner: Unassigned

PENDING CLAIMS AFTER ENTRY OF PRELIMINARY AMENDMENT

1. A method of inhibiting binding of a chaperone protein with its client protein or client polypeptide, wherein the method comprises contacting a chaperone protein with coumarin or a coumarin derivative, such that the coumarin or the coumarin derivative binds the chaperone protein, which binding inhibits the chaperone protein from binding its client protein or client polypeptide.

2. The method of claim 1, wherein the chaperone protein is heat shock protein (Hsp) 90.

3. The method of claim 1, wherein the coumarin or coumarin derivative is a coumarin antibiotic.

4. The method of claim 3, wherein the coumarin antibiotic is chlorobiocin or coumermycin A1.

5. The method of claim 3, wherein the coumarin antibiotic is novobiocin.

6. The method of claim 2, wherein the coumarin or coumarin derivative is novobiocin.

7. The method of claim 6, wherein novobiocin binds a carboxyl-terminal region of Hsp90.

8. The method of claim 1, wherein the client protein or the client polypeptide is a tyrosine or serine/threonine kinase.

9. The method of claim 8, wherein the client protein or the client polypeptide is tyrosine kinase $p185^{erbB2}$ or $p60^{v\text{-}src}$.

10. The method of claim 8, wherein the client protein or the client polypeptide is serine/threonine kinase Raf-1.

11. The method of claim 1, wherein the client protein or the client polypeptide is a mutated p53 protein.

12. The method of claim 1, wherein the client protein or the client polypeptide is inactive subsequent to binding of the chaperone protein to the coumarin or the coumarin derivative.

13. The method of claim 12, wherein the client protein or the client polypeptide is degraded.

14. The method of claim 1, wherein the chaperone protein is in a cell and cellular proliferation is inhibited.

15. The method of claim 14, wherein the cellular proliferation is cancer.

16. The method of claim 1, wherein the client protein is hepatitis B virus reverse transcriptase.

17. The method of claim 16, whereupon hepatitis B virus is inhibited.

18. The method of claim 1, wherein the client protein is a steroid hormone receptor.

19. The method of claim 18, wherein the effect of the steroid hormone receptor is modulated.

20. The method of claim 1, wherein the client protein is in a cell and is Hsf-1.

21. The method of claim 20, wherein the response of Hsf-1 to stress is inhibited.

22. The method of claim 1, which is *in vivo*.

23. The method of claim 1, which is *ex vivo*.

METHOD OF INHIBITING A CHAPERONE PROTEIN

TECHNICAL FIELD OF THE INVENTION

The present invention relates to a method of inhibiting binding of a chaperone protein with its client protein or client polypeptide.

BACKGROUND OF THE INVENTION

Chaperone proteins are proteins that mediate the correct assembly of other proteins or polypeptides, called client proteins or client polypeptides, but are not themselves part of the assembled structure. These proteins are involved in many macromolecular assembly processes, such as the folding and refolding of proteins during their synthesis and transport, as well as the association of polypeptides with each other and other macromolecules to form oligomeric complexes. They have been implicated in the folding of newly synthesized polypeptide chains, in the partial unfolding or disassociation that may occur when a protein carries out its function, in the transport of proteins across membranes, and in the repair of proteins partially denatured by exposure to environmental stresses, such as high temperature.

Chaperone proteins act by binding noncovalently to specific structural features in their target that are accessible only during assembly, and so inhibit unproductive assembly pathways that would otherwise act as kinetic dead ends, producing an incorrect, non-functional structure. Their function is required because many cellular processes involving protein assembly carry an inherent risk of malfunction as a result of the large number, variety, and flexibility of the noncovalent interactions that hold proteins in their functional conformations. A number of essential cellular processes involve the transient exposure of interactive protein surfaces to the intracellular environment with the consequent risk of errors due to incorrect interactions. Such processes include: protein synthesis, protein transport or translocation, protein function, organelle biogenesis, and stress response. Chaperone proteins are located in all parts of a cell where protein assembly occurs. Members of different classes of chaperone proteins cooperate in mediating such processes as the transport of polypeptides into mitochondria and their assembly into their final functional conformations.

Recently, it has become clear that chaperone proteins interact with a variety of proteins involved in cell proliferation. One such chaperone protein, heat shock protein (Hsp) 90 is constitutively expressed at 2-10 fold higher levels in tumor cells compared to their normal counterparts (see Ferrarini et al., *Int. J. Cancer* 51: 613-619 (1992)). Various compounds have been known to interfere with the chaperone protein

function of Hsp90. For example, the benzoquinone ansamycins, geldanamycin and herbimycin A, together with a non-related macrocyclic antibiotic, radicicol, have been shown to bind Hsp90 and to interfere with its function, including its function in tumor cell proliferation (see Whitesell et al., *Proc. Nat'l Acad. Sci. USA* 91: 8324-8328 (1994); Schulte et al., *Cell Stress and Chaperone Proteins* 3: 100-108 (1998); Johnson et al., *Mol. Endocrinol.* 9: 670-678 (1995); Sullivan et al., *J. Biol. Chem.* 272: 8007-8012 (1997)). Use of these compounds is not necessarily well-suited in clinical applications, as they display *in vivo* toxicity unrelated to their Hsp90 antagonism.

In view of the above, there exists a need for further methods utilizing chaperone protein antagonists. In particular, there is a need for methods utilizing clinically appropriate compounds that interfere with the function of chaperone proteins. The present invention provides such methods. These and other advantages of the present invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

BRIEF SUMMARY OF THE INVENTION

The present invention provides a method of inhibiting binding of a chaperone protein with its client protein or client polypeptide. The method comprises contacting a chaperone protein with a coumarin or a coumarin derivative, such that the coumarin or the coumarin derivative binds the chaperone protein, which inhibits the chaperone protein from binding its client protein or client polypeptide. The client protein or the client polypeptide is inactive or less active subsequent to binding of the chaperone protein to coumarin or the coumarin derivative.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method of inhibiting binding of a chaperone protein with its client protein or client polypeptide. The method comprises contacting a chaperone protein with a coumarin or a coumarin derivative, such that the coumarin or the coumarin derivative binds the chaperone protein, which inhibits the chaperone protein from binding its client protein or client polypeptide.

A chaperone protein in the context of the present invention can be any suitable chaperone protein or chaperone protein complex. Examples of chaperone and chaperone complex protein families include, but are not limited to, nucleoplasmins, chaperonins, heat shock proteins (Hsps), DnaJ protein, GrpE protein, SecB protein, signal recognition particle, prosequences, ubiquitinated proteins, PapD proteins, PrtM and PrsA, Lim protein, Rb protein, small heat shock proteins, ExbB protein, and prions.

Examples of heat shock protein complexes include, but are not limited to:

(1) the steroid receptor-associated Hsp90-containing intermediate folding complex, which functions in maturation of the progesterone receptor (PR) via an intermediate complex, Hsp70/Hsp90/$p60^{Hop}$-PR, such that, although the receptor is not yet competent to bind hormone, the intermediate complex is required for eventual maturation;

(2) the Hsp70 family of chaperone proteins, which includes the cytosolic chaperone protein Hsc70, the heat stress-induced Hsp70, and the endoplasmic reticulum chaperone protein Grp78;

(3) co-chaperone protein $p48^{Hip}$, which binds to Hsp70's adenosine triphosphatase (ATPase) domain and stabilizes its adenosine diphosphate (ADP)-bound state;

(4) $p60^{Hop}$, which binds Hsp70 via an N-terminal tetratricopeptide repeat (TPR) motif, which is a degenerate sequence of 34 amino acids that often occurs in tandem and appears to mediate protein-protein interaction, and to Hsp90 via a second TPR motif which is closer to the C-terminus and, when incubated with purified Hsp70 and Hsp90, forms a tripartite complex with the other two proteins;

(5) p23, which is a highly acidic phosphoprotein that is a component of the mature PR complex, is often found associated with Hsp90 and an immunophilin, even in the absence of PR, and, in addition to participating in steroid receptor-associated Hsp90 complexes, also can be found in Hsp90 complexes with hepatitis B virus reverse transcriptase, mutated p53, aryl hydrocarbon receptor, and heat shock transcription factor Hsf-1;

(6) immunophilins, which bind to Hsp90 via their TPR motifs and replace $p60^{Hop}$ in the multichaperone protein complex, and also may have a transport function, since they have been found associated with both microtubules and nuclear structures;

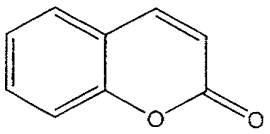
(7) two other proteins containing TPR motifs that have been shown to bind to Hsp90, namely PP5, which is a serine/threonine phosphatase that contains 4 TPR motifs in its N-terminal domain and Mas70p, which is a component of the protein import machinery in the outer mitochondrial membrane that contains 7 TPR motifs (the existence of a Mas70p-Hsp90 complex suggests that Hsp90 may participate in protein movement into mitochondria); and

(8) $p50^{Cdc37}$, which replaces immunophilin in Hsp90 complexes with kinases such as Cdk4, v-Src and Raf-1 and does not possess TPR motifs, but binds to a site close to Hsp90's TPR binding domain.

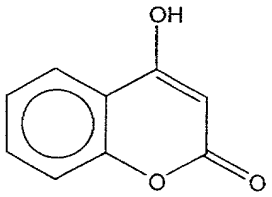
Preferably, the chaperone protein is Hsp90, which is one of the most abundant proteins in eukaryotic cells, comprising 1-2% of total cellular protein, even under non-stress conditions. In mammalian cells, there are two Hsp90 isomers in the cytosol, Hsp90α and Hsp90β in humans and Hsp86 and Hsp84 in mice, with a third homologue, glucose regulated protein 94 (Grp94), localized primarily in the endoplasmic reticulum. An additional truncated, cytosolic member of the family, designated Hsp75, has recently been identified. Functional analysis has revealed that Hsp90 is composed of three primary domains: well-conserved amino and carboxyl terminal regions separated by a charged domain (see Scheibel et al., *J. Biol. Chem.*, 272:18608-18613 (1997); Scheibel et al., *Proc Natl Acad Sci U S A* 95:1495-1499 (1998)).

Hsp90 is required for full activity of several "ligand-dependent" transcription factors, including members of the steroid receptor family, the aryl hydrocarbon receptor and the retinoid receptor. "Ligand-independent" transcription factors that bind Hsp90 include MyoD, Hsf-1, mutated p53 and hypoxia inducible factor 1-α. Hsp90 or Grp94 also has been demonstrated to be necessary for proper function and correct cellular localization of a wide variety of tyrosine and serine/threonine kinases, including members of the Src family, oncogenic epidermal growth factor (EGF) receptor-related tyrosine kinase $p185^{erbB2}$, cyclin-dependent kinase 4 (Cdk4), cell cycle-associated kinase Wee1, and serine-threonine kinase Raf-1, which is a member of the mitogen-activated protein (MAP) kinase pathway.

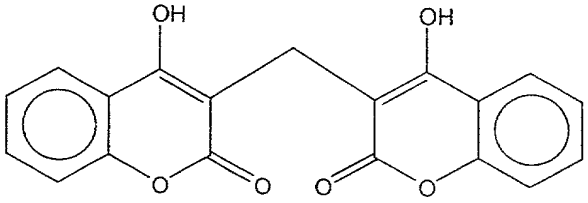
The "coumarin or a coumarin derivative" is any suitable member of the coumarin family. This family of compounds has a 2*H*-1-benzopyran-2-one core (molecular formula $C_9H_6O_2$), which is shown below (see DeGarmo, *Coumarin, in* ECT 1st ed., vol. 4, 588-593; 2nd ed., vol. 6, 425-433, (Monsanto Chemical Co.); Sethna et al., *Chem. Rev.* 36: 27 (1945)). Derivatives include, e.g., alkyl, hydroxy, and methoxy derivatives, as well as more complicated derivatives, such as, e.g., 3,4-dihydrocoumarin, 6-methylcoumarin, umbelliferone (7-hydroxycoumarin), 4-hydroxycoumarin (shown below), dicumarol (shown below), warfarin (3-substituted 4-hydroxycoumarins, an example of which is shown below), phenprocoumon (shown below), coumarone (benzofuran), and coumarone-indene resins.



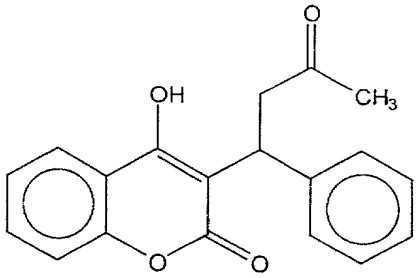
Coumarin



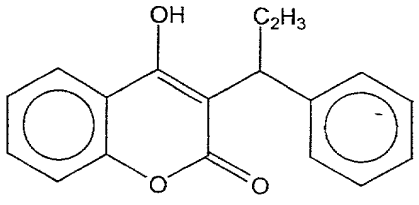
4-Hydroxycoumarin



Dicumarol

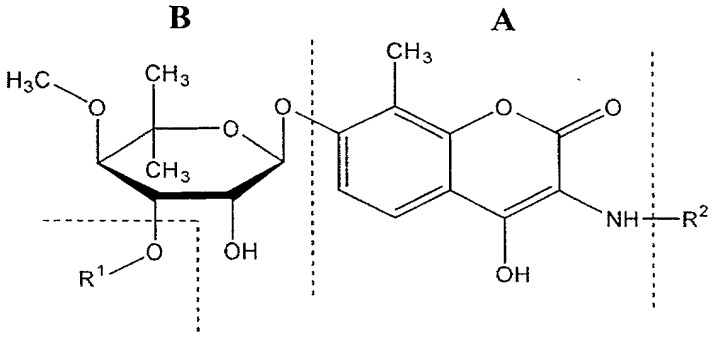


Warfarin



Phenprocoumon

Preferably, coumarin or a coumarin derivative is a coumarin antibiotic. Coumarin antibiotics have a 4-hydroxy-8-methylcoumarin core (A) and a noviose sugar moiety (B), shown below (see Finland & Nichols, *Anti-Biot. Chemother.* 4: 1954-68 (1957); Godfrey & Price, *Structure-Activity Relationships in Coumermycins*, *in* Structure-Activity Among the Semisynthetic Antibiotics 653-718 (D. Perlman, ed. 1977)).



R^1 and R^2 can be any suitable substituent. Examples of suitable substituents for R^1 include H, alkyl, haloalkyl, alkylthio, alkenyl, alkynyl, alkoxy, haloalkoxy, alkylamino, dialkylamino, cycloalkyl, aryl, aralkyl, heterocycloalkyl, heteroaryl, or O=C–R^3. R^3 can be any suitably substituent, e.g., H, alkyl, haloalkyl, alkylthio, alkenyl, alkynyl, alkoxy, haloalkoxy, alkylamino, dialkylamino, cycloalkyl, aryl, aralkyl, heterocycloalkyl, heteroaryl, or NR^4. R^4 can be any suitable substituent, e.g., H, alkyl, haloalkyl, alkylthio, alkenyl, alkynyl, alkoxy, haloalkoxy, alkylamino, dialkylamino, cycloalkyl, aryl, aralkyl, heterocycloalkyl, or heteroaryl. Preferably, R^3 is a substituted heteroaryl. Examples of suitable substituents for R^2 include H, alkyl, haloalkyl, alkylthio, alkenyl, alkynyl, alkoxy, haloalkoxy, alkylamino, dialkylamino, cycloalkyl, aryl, aralkyl, heterocycloalkyl, heteroaryl, or O=C–R^5. R^5 can be any suitable substituent, e.g., H, alkyl, haloalkyl, alkylthio, alkenyl, alkynyl, alkoxy, haloalkoxy, alkylamino, dialkylamino, cycloalkyl, aryl, aralkyl, heterocycloalkyl, or heteroaryl. Preferably, R^5 is an aryl, more preferably, monocyclic, and most preferably, 6-membered and substituted with, e.g., halogen (e.g., fluorine, chlorine, or bromine), alkyl, haloalkyl, alkenyl, alkynyl, alkoxy, haloalkoxy, nitro, cyano, hydroxy, amino, thio, alkylthio, or acyl. These substituents may, themselves, be substituted or unsubstituted.

As utilized herein, the term "alkyl" means a straight-chain or branched-chain alkyl radical which, unless otherwise specified, contains from about 1 to about 20 carbon atoms chain, preferably from about 1 to about 10 carbon atoms, more preferably from about 1 to about 8 carbon atoms, and most preferably from about 1 to about 6

carbon atoms. Examples of such alkyl radicals include methyl, ethyl, propyl (i.e., *n*- or isopropyl), butyl (i.e., *n*-, *sec*-, iso, or *tert*-butyl), pentyl, isoamyl, hexyl, octyl, dodecanyl, and the like.

The term "haloalkyl" means alkyl, as defined herein, wherein a hydrogen atom is replaced by a halogen (e.g., chlorine, fluorine, or bromine).

The term "alkylthio" means alkyl, as defined herein, which has a sulfur substituent. Example of alkylthios include methanethiol, ethanethiol, and the like.

The term "alkenyl" means a straight-chain or branched-chain alkenyl radical, which has one or more double bonds and, unless otherwise specified, contains from about 2 to about 20 carbon atoms, preferably from about 2 to about 10 carbon atoms, more preferably from about 2 to about 8 carbon atoms, and most preferably from about 2 to about 6 carbon atoms. Examples of alkenyl radicals include vinyl, allyl, 1,4-butadienyl, isopropenyl, and the like.

The term "alkynyl" means a straight-chain or branched-chain alkynyl radical, which has one or more triple bonds and contains from about 2 to about 20 carbon atoms, preferably from about 2 to about 10 carbon atoms, more preferably from about 2 to about 8 carbon atoms, and most preferably from about 2 to about 6 carbon atoms. Examples of alkynyl radicals include ethynyl, propynyl (propargyl), butynyl, and the like.

The term "alkoxy" means a straight-chain or branched-chain alkoxy radical, which has one or more ether groups of the general formula O–R and contains from about 2 to about 20 carbon atoms, preferably from about 2 to about 10 carbon atoms, more preferably from about 2 to about 8 carbon atoms, and most preferably from about 2 to about 6 carbon atoms. Examples of alkoxy radicals include methoxy, ethoxy, and the like.

The term "haloalkoxy" means alkoxy, as defined herein, wherein a hydrogen atom is replaced by a halogen (e.g., chlorine, fluorine, or bromine).

The terms "alkylamino" and "dialkylamino" mean an alkyl amine or a dialkyl amine radical, wherein the term "alkyl" is defined as above. Examples of alkylamino radicals include methylamino ($NHCH_3$), ethylamino ($NHCH_2CH_3$), propylamino (i.e., *n*- or isopropylamino), butylamino (i.e., *n*-, iso, *sec*-, or *tert*-butylamino), *n*-hexylamino, and the like. Examples of dialkylamino radicals include dimethylamino ($N(CH_3)_2$), diethylamino ($N(CH_2CH_3)_2$), dipropylamino (i.e., di-*n*- or di-isopropylamino), dibutylamino (i.e., di-*n*-, di-iso, di-*sec*-, or di-*tert*-butylamino), di-*n*-hexylamino, and the like.

The term "cycloalkyl" means a monocyclic alkyl radical, or a polycyclic alkyl which comprises one or more alkyl carbocyclic rings, which can be the same or

different when the polycyclic radical has 3 to about 10 carbon atoms in the carbocyclic skeleton of each ring. Preferably, the cycloalkyl has from about 4 to about 7 carbon atoms, more preferably from about 5 to about 6 carbons atoms. Examples of monocyclic cycloalkyl radicals include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclodecyl, and the like. Examples of polycyclic cycloalkyl radicals include decahydronaphthyl, bicyclo[5.4.0]undecyl, adamantyl, and the like.

The term "aryl" refers to an aromatic carbocyclic radical, as commonly understood in the art, and includes monocyclic and polycyclic aromatics such as, e.g., phenyl and naphthyl radicals, which radicals are, unless indicated otherwise, optionally substituted with one or more substituents selected from the group consisting of a halogen, an alkyl, alkoxy, amino, cyano, nitro, and the like. Preferably, the aryl has one or more six-membered carbocyclic rings including, e.g., phenyl, naphthyl, and biphenyl, and are optionally substituted as set forth herein.

The term "aralkyl" means alkyl as defined herein, wherein an alkyl hydrogen atom is replaced by an aryl as defined herein. Examples of aralkyl radicals include benzyl, phenethyl, 1-phenylpropyl, 2-phenylpropyl, 3-phenylpropyl, 1- naphthylpropyl, 2- naphthylpropyl, 3- naphthylpropyl, 3-naphthylbutyl, and the like.

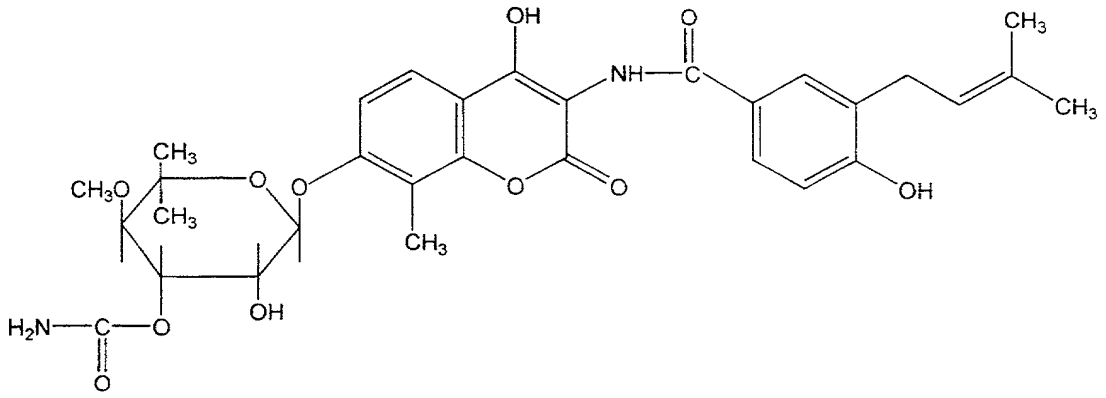
The terms heterocycle and heterocyclic refers to both heterocycloalkyls and heteroaryls. The term "heterocycloalkyl" means a cycloalkyl radical as defined herein (including polycyclics), wherein at least one carbon of a carbocyclic ring is substituted with a heteroatom such as, e.g., O, N, or S. The heterocycloalkyl optionally has one or more double bonds within a ring, but is not necessarily aromatic. The heterocycloalkyl preferably has 3 to about 10 atoms (members) in the carbocyclic skeleton of each ring, preferably from about 4 to about 7 atoms, more preferably from about 5 to about 6 atoms. Examples of heterocycloalkyl radicals include epoxy, aziridyl, oxetanyl, tetrahydrofuranyl, ribose, dihydrofuranyl, piperidinyl, piperazinyl, pyranyl, morpholinyl, and the like.

The term "heteroaryl" means a radical defined by an aromatic heterocyclic ring as commonly understood in the art, including monocyclic radicals such as, e.g., imidazole, thiazole, pyrazole, pyrrole, furane, pyrazoline, thiophene, oxazole, isoxazole, pyridine, pyridone, pyrimidine, cytosine, 5-methylcytosine, thymine, pyrazine, and triazine radicals, and polycyclics such as, e.g., quinoline, isoquinoline, indole, purine, adenine, guanine, N^6-methyladenine, and benzothiazole radicals, which heteroaryl radicals are optionally substituted with one or more substituents selected from the group consisting of a halogen, an alkyl, alkoxy, an amino, a cyano, a nitro, and the like. It will also be appreciated that heteroaryls, as defined herein, are not necessarily "aromatic" in the same context as phenyl is aromatic, although heteroaryls

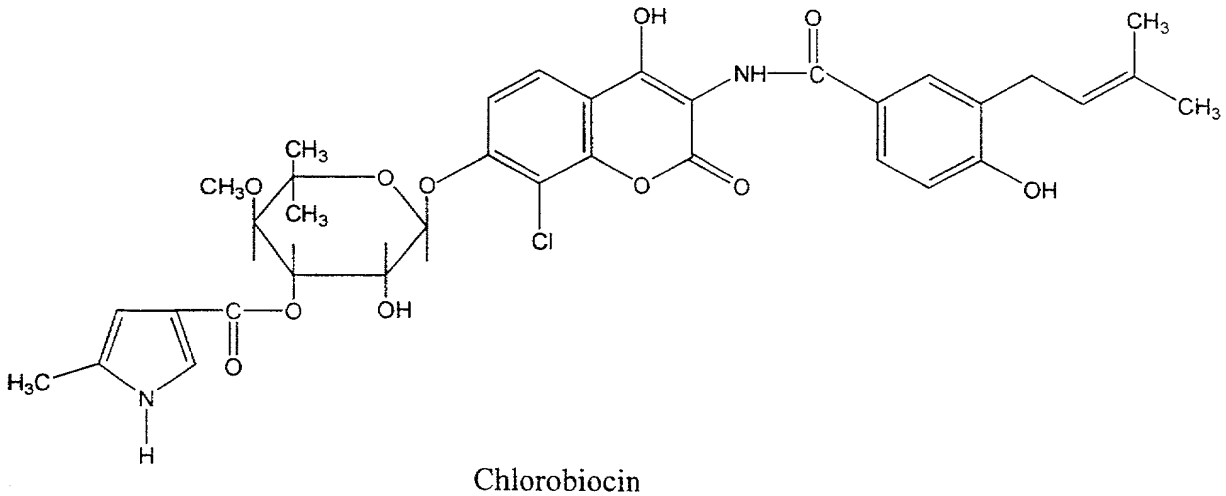
nonetheless demonstrate physical and chemical properties associated with aromaticity, as the term is understood in the art.

Any of the above may be substituted or unsubstituted with any suitable substituent. Examples of suitable substituents include halogen (e.g., fluorine, chlorine, or bromine), alkyl, haloalkyl, alkenyl, alkynyl, alkoxy, haloalkoxy, nitro, cyano, hydroxy, amino, thio, alkylthio, or acyl. These substituents may, themselves, be substituted or unsubstituted.

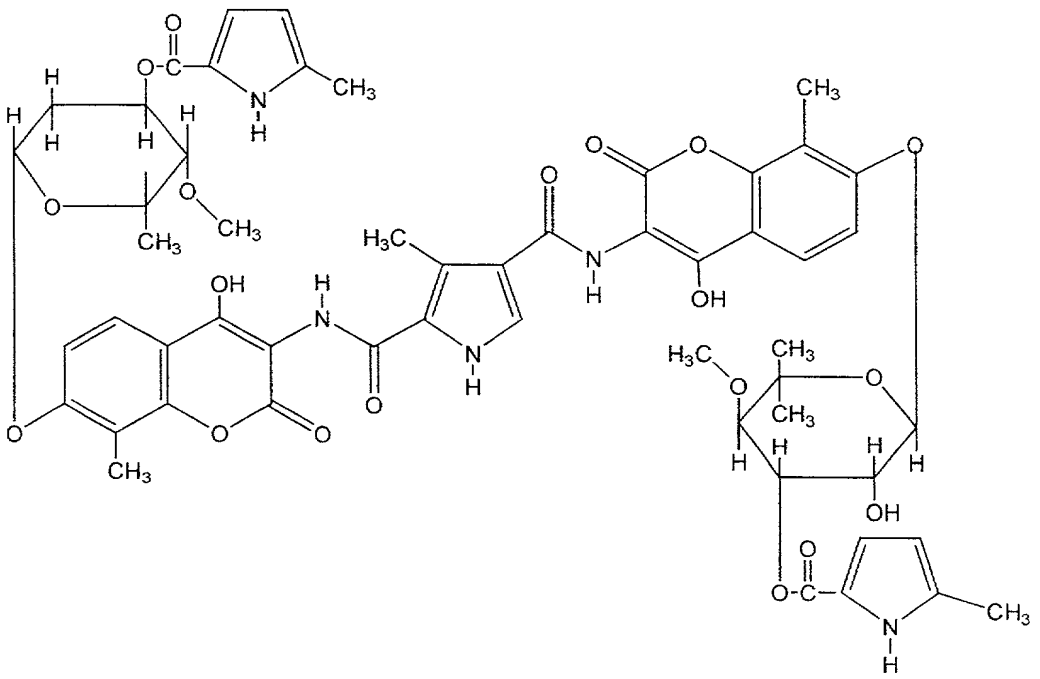
More preferably, the coumarin antibiotic is novobiocin, chlorobiocin, or coumermycin A1, which are shown below.



Novobiocin



Chlorobiocin



Coumermycin A1

Most preferably, the coumarin antibiotic is novobiocin, a well-studied antibiotic whose pharmokinetics and toxicity profile are clearly understood. Doses of 4 g/day (well below the maximum tolerated dose) yield a plasma level ≥ 200-300 μg/ml, 2 hrs after post-administration, corresponding to a 300-500 μM drug concentration (see Drusano et al., *Antimicrob. Agents Chemother.* 30: 42-45 (1986); Eder et al., *J. Clin. Invest.* 79: 1524-1528 (1987)).

The contacting of coumarin or a coumarin derivative with the chaperone protein can be carried out in any suitable manner. For example, coumarin or a coumarin derivative can be contacted with the chaperone protein by *in vivo* or *ex vivo* administration. Preferably, coumarin or a coumarin derivative is administered *in vivo* to a mammal, more preferably, coumarin or a coumarin derivative is administered *in vivo* to a human.

To facilitate administration of coumarin or a coumarin derivative, both *in vivo* and *ex vivo*, it can be formulated into a suitable composition. Generally, such compositions (e.g., pharmaceutical compositions) include the active ingredient (i.e., coumarin or a coumarin derivative) and an acceptable carrier (e.g. a pharmacologically acceptable carrier). Such compositions can be suitable for delivery of the active ingredient to a patient for medical application, and can be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving,

granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention can be formulated in a conventional manner using one or more pharmacologically (e.g., physiologically) acceptable carriers comprising excipients, as well as optional auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. Thus, for injection, the active ingredient can be formulated in aqueous solutions, preferably in physiologically compatible buffers. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art. For oral administration, the active ingredient can be combined with carriers suitable for inclusion into tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like. For administration by inhalation, the active ingredient is conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant. The active ingredient can be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Such compositions can take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Other pharmacological excipients are known in the art.

The coumarin or coumarin derivative can be administered in unit dosage form, such as a tablet or capsule. The term "unit dosage form" as used herein refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity, alone or in combination with other active agents, calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier, or vehicle. The specifications for the unit dosage forms of the present invention depend on the particular coumarin or coumarin derivative employed and the effect to be achieved, as well as the pharmacodynamics associated with each in the host. The dose administered should be an "effective amount" or an amount necessary to achieve an "effective level" in the individual patient. Since the effective level is used as the preferred endpoint for dosing, the actual dose and schedule can vary, depending on interindividual differences in pharmacokinetics, drug distribution, and metabolism.

One skilled in the art can easily determine the appropriate dose, schedule, and method of administration for the exact formulation of the composition being used, in order to achieve the desired effective level in the individual patient. Many of the

coumarin or coumarin derivatives are well-know compounds with clearly established effective amounts required to achieve effective levels. However, one skilled in the art also can readily determine and use an appropriate indicator of the effective level of the compounds of the present invention by a direct (e.g., analytical chemical analysis) or indirect analysis of appropriate patient samples (e.g., blood and/or tissues) to determine the suitable dosage required for any given coumarin or coumarin derivative administered.

The interaction between the chaperone protein and coumarin or a coumarin derivative is such that the chaperone protein does not bind or binds with less affinity to its client protein or client polypeptide. Such interference with binding can be accomplished by any suitable method. For example, the chaperone protein and coumarin or a coumarin derivative may interact by binding covalently or non-covalently. If non-covalent, the binding can be through hydrogen bonding, ionic bonding, hydrophobic or van der Waals interactions, or any other appropriate type of binding. Preferably, the binding is through non-covalent binding, more preferably, hydrogen or hydrophobic binding. In the preferred embodiment wherein the chaperone protein is Hsp90 and the coumarin or coumarin derivative is novobiocin, interaction is such that novobiocin binds a carboxyl-terminal region of Hsp90, which contains an adenosine triphosphate (ATP)-binding domain.

A client protein or client polypeptide in the context of the present invention can be any suitable client protein or client polypeptide. Client proteins or client polypeptides include, but are not limited to, various transcription factors, including steroid hormone receptors, aryl hydrocarbon receptor, v-ErbA, retinoid receptor, Sim, Myo D1, Hsf-1, mutated p53, various protein kinases, and various other proteins, such as cytoskeletal proteins, calmodulin, G protein βγ-subunits, Proteasome, Hepatitis B virus reverse transcriptase, tumor necrosis factor (TNF) receptors and retinoblastoma protein. *See generally* Thormeyer & Baniahmad, *Int. J. Mol. Med.* 4(4):351-58 (1999) (vErbA); Moffett & Pelletier, *FEBS Lett.* 466(1):80-6 (2000) (Sim); Jones et al., *B. Biol. Sci.* 326(1235):277-84 (1990) (Myo D1); Green et al., *Mol. Cell Biol.* 15:3354-62 (1995) (Hsf-1). Examples of steroid hormone receptors include the glucocorticoid receptor, the progesterone receptor, the estrogen receptor, the androgen receptor, and the mineralocorticoid receptor. Cytoskeletal proteins include actin, tubulin, and intermediate filaments.

Preferably, the client protein or the client polypeptide is a protein kinase. More preferably, the protein kinase is a tyrosine kinase or a serine/threonine kinase. Serine/Threonine kinases include the Raf family of kinases, MEK (MAP/ERK (extracellular signal-regulated kinase)), heme regulated E1F-2a kinase, calmodulin

dependent eukaryotic elongation factor (eEF)-2 kinase, and casein kinase. *See generally* Weber et al., *Oncogene* 19(2):169-76 (2000) (Raf family); Bommhardt et al., *J Immunol.* 164(5):2326-37 (2000) (MEK). Tyrosine kinases include HER-2/neu- (also known as c-erbB2) encoded p185 ($p185^{erbB2}$), the Src family kinases ($p60^{v\text{-}src}$ or $p60^{c\text{-}src}$), Wee1, Sevenless, and Fps/Fes. *See generally* Hung & Lau, *Semin. Oncol.* 26 (4 Suppl 12):51-9 (1999) (HER-2/neu-encoded p185); Schwartzberg, *Oncogene* 17:1463-8 (1998) (Src family kinases); Pendergast, *Curr. Opn. Cell Biol.* 8(2):174-81 (1996) (Wee1); Smithgall et al., *Crit. Rev. Oncogene* 9(1):43-62 (1998) (Fps/Fes). Most preferably, the client protein or the client polypeptide is $p185^{erbB2}$, $p60^{v\text{-}src}$ or Raf-1. Alternatively, the client protein or the client polypeptide is preferably a mutated p53 protein.

After binding of the chaperone protein to the coumarin or the coumarin derivative, the client protein or the client polypeptide is generally inactive or less active. The client protein or the client polypeptide can be inactive or less active through any suitable method. For example, the client protein or the client polypeptide can be degraded by cellular machinery because it is not coupled with its chaperone protein, or it can have an incorrect conformation that distorts its active or recognition site and interferes with the polypeptide performing its designated function. The client protein or the client polypeptide can also, e.g., be located in an inappropriate area of the cell, which prevents it from performing its designated function. Preferably, the client protein or client polypeptide is degradation.

The present invention is further described in the following examples. These examples serve only to illustrate the invention and are not intended to limit the scope of the invention in any way.

EXAMPLES

Example 1

This example demonstrates binding of a chaperone protein to coumarin or a coumarin derivative. In addition, this example illustrates that the incubation of the chaperone protein with various coumarin derivatives inhibits subsequent binding of the chaperone protein to its client protein or client polypeptide.

To demonstrate binding of a chaperone protein to novobiocin (a coumarin antibiotic), the novobiocin was first immobilized on sepharose (see Staudenbauer & Orr, *Nucleic Acids Res.* 9: 3589-3603 (1981)). A chaperone protein, either pure Hsp90, or a solution containing Hsp90 in a cell lysate, was subsequently incubated with the immobilized novobiocin. The cell lysate was preincubated with various members of the coumarin family of antibiotics, namely novobiocin, chlorobiocin, or coumermycin A1,

and also ATP to determine their ability to inhibit binding of the Hsp90. The amount of Hsp90 bound to the immobilized novobiocin was analyzed by SDS-PAGE followed by silver staining using, e.g., a commercially available kit from BioRad or Western blotting with appropriate antibodies, e.g., SPA830 (StressGen Biotechnology, Vancouver, Canada).

Immobilized novobiocin bound in a hydrophobic manner to both of the pure Hsp90 and the Hsp90 present in cell lysate. Pre-incubation of the cell lysate with excess soluble novobiocin, chlorobiocin, coumermycin A1, or ATP inhibited, in a dose-dependent manner, subsequent Hsp90 binding to immobilized novobiocin, as determined by Western blotting. Soluble novobiocin inhibited Hsp90 binding to immobilized novobiocin at about 8 mM. Chlorobiocin and coumermycin A1 inhibited Hsp90 binding to immobilized novobiocin at about 0.5 mM, while ATP inhibited Hsp90 binding between about 10 and 15 mM, as demonstrated by silver staining.

These data demonstrate that novobiocin, a coumarin derivative, binds to chaperone proteins such as Hsp90 and that subsequent Hsp90-binding can be inhibited by contact with the coumarin derivatives novobiocin, chlorobiocin, and coumermycin A1, as well as ATP.

Example 2

This example demonstrates *in vivo* depletion of client protein or client polypeptide through contact of a coumarin or a coumarin derivative with a chaperone protein.

To determine the depletion of Hsp90 client polypeptides, commercially available SKBR3 cells (American Type Culture Collection, Rockville, MD) and *v-src*-3T3 fibroblasts (National Cancer Institute, Rockville, MD) were treated with increasing concentrations of novobiocin, a coumarin antibiotic. The levels of the various Hsp90 client polypeptides were assayed by Western blotting: the membranes were first probed with an appropriate primary antibody (i.e., $p185^{erbB2}$, $pp60^{v\text{-}src}$, and p53 antibodies, Oncogene Research Products/Calbiochem, Cambridge, MA; gelsolin antibody, Sigma; Grp78 and Raf-1 antibodies, Santa Cruz Biotechnology, Santa Cruz, CA; and scinderin antibody, a gift from Dr. J.M. Trifaró, University of Ottawa, Canada), followed by a secondary antibody conjugated to horseradish peroxidase, and the signal detected by chemiluminescent reagents.

As a control, the steady-state levels of scinderin, an actin-associated protein, and BiP (Grp78), an Hsp70 family chaperone protein localized to the endoplasmic reticulum, were assayed using the same method described above. General interference

with protein synthesis was also determined after overnight cyclohexamide treatment of SKBR3 cells.

The level of $p185^{erbB2}$ protein, an Hsp90 client polypeptide, was reduced by 80% relative to untreated controls after overnight treatment with 800 μM novobiocin, and by 40% compared to controls after overnight treatment with 300 μM novobiocin. Hsp90 client polypeptides Raf-1, $p60^{v\text{-}src}$, and mutant p53 protein levels were also significantly reduced after overnight treatment with novobiocin. In v-src-transformed 3T3 fibroblasts, the level of $p60^{v\text{-}src}$ protein was reduced by 50% after exposure to 500 μM novobiocin. Novobiocin also significantly depleted mutant p53 protein in SKBR3 cells, while depleting Raf-1 protein to an undetectable level in the same cells. As a control, the steady-state levels of scinderin, an actin-associated protein, and BiP (Grp78), an Hsp70 family chaperone protein localized to the ER, were not altered by the doses and exposure times of novobiocin used. General interference with protein synthesis is also not likely since overnight cyclohexamide treatment of SKBR3 cells did not significantly affect the steady state levels of the proteins affected by novobiocin.

Thus, these data demonstrate that contact of novobiocin, a coumarin derivative, with the chaperone protein Hsp90 inhibits Hsp90-binding with $p185^{erbB2}$, an Hsp90 client protein, thereby resulting in the subsequent inactivation, and specifically depletion, of the client protein $p185^{erbB2}$.

Example 3

This example demonstrates that contacting coumarin or a coumarin derivative with a chaperone protein decreases client protein or client polypeptide levels in normal human peripheral blood mononuclear cells (PBMC).

To determine the decrease in Raf-1 (an Hsp90 client protein) after treatment with novobiocin (a coumarin antibiotic), PBMC were isolated from human blood and cultured, according to standard protocol, with varying concentrations of novobiocin for 14 hrs. Levels of Raf-1 protein were determined using Western blotting, as described in Example 2. A control protein, gelsolin, was also assayed using the same method. Cell viability was assessed by the ability of cells to take up a vital stain (e.g., trypan blue), which is ordinarily excluded by viable cells.

The Hsp90 client Raf-1 protein was found to be depleted in a dose-dependent manner by incubation with novobiocin, a coumarin derivative, such that it was almost completely depleted at a concentration of about 0.8 mM novobiocin. Other protein levels remained unaltered, even at the highest drug concentration tested. PBMC remained viable, as assayed by trypan blue.

Example 4

This example demonstrates that *in vivo* administration of coumarin or a coumarin derivative affects client protein or client polypeptide levels in murine splenocytes.

To determine the level of Raf-1 protein (an Hsp-90 client polypeptide), normal C57Bl6 mice received intraperitoneal injections (100 mg/kg) of novobiocin (a coumarin antibiotic) at 12 hr intervals for 5 days. Animals were sacrificed 3 hrs after the last injection. The spleens were removed, cells were lysed and total protein was assayed by the Bradford method using, e.g., a commercially available kit from BioRad. At this dose, the serum level of novobiocin has been reported to vary between 100 and 450 µg/ml during the first hr, with the plasma clearance half-life of the drug approximately 80 min. in mice (see Eder at al., *Cancer Res.* 51: 510-513 (1991)). Raf-1 levels were determined by Western blotting, as described in Example 2. A control protein, gelsolin, was also assayed using the same method. Optical density of the Raf-1 specific bands was determined using NIH Image software.

After 5 days on this regimen, mice receiving novobiocin had significantly lower levels of splenocyte Raf-1 protein than did controls. On average, Raf-1 protein in the novobiocin-treated group was reduced by 44% as compared to controls. In 7 of 10 treated mice, the mean splenocyte Raf-1 protein level was reduced even further, to 29% of control. Splenocyte gelsolin remained unchanged in all treated mice.

These data demonstrate that *in vivo* treatment with novobiocin significantly reduces the levels of the client protein Raf-1 in the tissue targeted. Presumably, the reduced levels were due to contact between novobiocin, a coumarin derivative, and the chaperone protein Hsp90.

All of the references cited herein, including patents, patent applications, and publications, are hereby incorporated in their entireties by reference.

While this invention has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations of the preferred embodiments may be used and that it is intended that the invention may be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the following claims.

WHAT IS CLAIMED IS:

1. A method of inhibiting binding of a chaperone protein with its client protein or client polypeptide, wherein the method comprises contacting a chaperone protein with coumarin or a coumarin derivative, such that the coumarin or the coumarin derivative binds the chaperone protein, which binding inhibits the chaperone protein from binding its client protein or client polypeptide.

2. The method of claim 1, wherein the chaperone protein is heat shock protein (Hsp) 90.

3. The method of claim 1, wherein the coumarin or coumarin derivative is a coumarin antibiotic.

4. The method of claim 3, wherein the coumarin antibiotic is chlorobiocin or coumermycin A1.

5. The method of claim 3, wherein the coumarin antibiotic is novobiocin.

6. The method of claim 2, wherein the coumarin or coumarin derivative is novobiocin.

7. The method of claim 6, wherein novobiocin binds a carboxyl-terminal region of Hsp90.

8. The method of claim 1, wherein the client protein or the client polypeptide is a tyrosine or serine/threonine kinase.

9. The method of claim 8, wherein the client protein or the client polypeptide is tyrosine kinase $p185^{erbB2}$ or $p60^{v\text{-}src}$.

10. The method of claim 8, wherein the client protein or the client polypeptide is serine/threonine kinase Raf-1.

11. The method of claim 1, wherein the client protein or the client polypeptide is a mutated p53 protein.

12. The method of claim 1, wherein the client protein or the client polypeptide is inactive subsequent to binding of the chaperone protein to the coumarin or the coumarin derivative.

13. The method of claim 12, wherein the client protein or the client polypeptide is degraded.

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

This declaration is of the following type:

☐ original ☐ design ☐ supplemental
☒ national stage of PCT
☐ divisional ☐ continuation ☐ continuation-in-part

My residence, post office address, and citizenship are as stated below next to my name. I believe I am the original, first, and sole inventor *(if only one name is listed below)* or an original, first, and joint inventor *(if plural names are listed below)* of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD OF INHIBITING A CHAPERONE PROTEIN

the specification of which:

☐ is attached hereto.
☒ was filed on September 12, 2001 as Application No. 09/936,449 and was amended on *(if applicable)*.
☐ was filed by Express Mail No. *as Application No. not known yet*, and was amended on *(if applicable)*.
☐ was filed on as PCT International Application No. PCT/ and was amended on *(if any)*.

I state that I have reviewed and understand the contents of the specification identified above, including the claim(s), as amended by any amendment referred to above.

I acknowledge the duty to disclose information that is material to the patentability of the application identified above in accordance with 37 CFR 1.56.

I claim foreign priority benefits under 35 USC 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate or 365(a) of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent, utility model, design registration, or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter and having a filing date before that of the application(s) from which the benefit of priority is claimed.

PRIOR FOREIGN PATENT, UTILITY MODEL, AND DESIGN REGISTRATION APPLICATIONS						
COUNTRY	PRIOR FOREIGN APPLICATION NO.	DATE OF FILING (day,month,year)	PRIORITY CLAIMED			
				YES		NO
				YES		NO
				YES		NO

I claim the benefit pursuant to 35 USC 119(e) of the following United States provisional patent application(s):

PRIOR U.S. PROVISIONAL PATENT APPLICATIONS, BENEFIT CLAIMED UNDER 35 USC 119(e)	
APPLICATION NO.	DATE OF FILING (day,month,year)
60/124,135	12 March 1999

I claim the benefit pursuant to 35 USC 120 of any United States patent application(s) or PCT international application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this patent application is not disclosed in the prior patent application(s) in the manner provided by the first paragraph of 35 USC 112, I acknowledge the duty to disclose material information as defined in 37 CFR 1.56 effective between the filing date of the prior patent application(s) and the national or PCT international filing date of this patent application.

PRIOR U.S. PATENT APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S., BENEFIT CLAIMED UNDER 35 USC 120					
U.S. PATENT APPLICATIONS			Status *(check one)*		
U.S. APPLICATION NO.	U.S. FILING DATE		PATENTED	PENDING	ABANDONED
1.					
2.					
3.					
PCT APPLICATIONS DESIGNATING THE U.S.			Status *(check one)*		
PCT APPLICATION NO.	PCT FILING DATE (day,month,year)	U.S. APPLICATION NOS. ASSIGNED (if any)	PATENTED	PENDING	ABANDONED
4. PCT/US00/06482	10 March 2000			X	
5.					
6.					

DETAILS OF FOREIGN APPLICATIONS FROM WHICH PRIORITY CLAIMED UNDER 35 USC 119 FOR ABOVE LISTED U.S./PCT APPLICATIONS				
ABOVE APPLICATION. NO.	COUNTRY	APPLICATION NO.	DATE OF FILING (day,month,year)	DATE OF ISSUE (day,month,year)
1.				
2.				
3.				
4.				
5.				
6.				

In re Appln. of Marcu et al.
Attorney Docket No. 213373

As a named inventor, I hereby appoint the following attorneys to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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Please recognize Leydig, Voit, & Mayer, Ltd. as Associate Attorneys in this case: Customer Number 23460.

23460

PATENT TRADEMARK OFFICE

I further direct that correspondence concerning this application be directed to Leydig, Voit & Mayer, Ltd.: Customer Number 23460.

23460

PATENT TRADEMARK OFFICE

I declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Attorney Docket No. 213373

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